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Antioxidants associated with fruit senescence and human health: Novel orange-fleshed non-netted honey dew melon genotype comparisons following different seasonal productions and cold storage durations

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Abstract

Orange-fleshed cantaloupe fruit (*Cucumis melo* L. Reticulatus group) continues to raise food-safety concerns due to attachment of enteric bacteria to sites on the netted surface inaccessible to sanitation. Non-netted orange-fleshed honey dew fruit (*Cucumis melo* L. Inodorus group) versus cantaloupe offers a safer and a healthier (nutritional content) option. Some commercially available non-netted orange-fleshed honey dew genotype fruit were compared for antioxidants associated with storage quality following autumn and spring production cycles, harvested at abscission (mature) and stored for up to 24 d at 5 °C or 10 °C. Spring versus autumn production generally yielded higher overall levels of 5-methyltetrahydrofolate (folic acid), calcium, malondialdehyde (MDA), and lipophilic total antioxidant capacities. 'Orange Delight' and 'Orange Dew', were generally superior to 'Honey Gold', 'Temptation' and a breeding line as they consistently demonstrated some of the highest levels of total ascorbic acid, β-carotene, and potassium. 'Orange Delight' and 'Orange Dew' were also among the cultivars with the highest activities of ascorbate peroxidase (EC 1.11.1.11), catalase (EC 1.11.1.6), and superoxide dismutase (EC 1.15.1.1). These two cultivars also exhibited the least increase in MDA (i.e. lipid peroxidation) during storage, suggesting antioxidant levels limited oxidative-related senescence compared to the other genotypes. Results indicate that there are significant differences in human health-related and storage quality-related phytochemical profiles between orange-fleshed honey dew cultivars and that high antioxidant levels are associated with reduced lipid peroxidation during fruit cold storage.
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Keywords: *Cucumis melo*; Ascorbate peroxidase; Catalase; Malondialdehyde; 5-Methyltetrahydrofolate; Superoxide dismutase

1. Introduction

Sweet melons, including netted cantaloupe (*Cucumis melo* L. Reticulatus group), watermelon (*Citrullus lanatus* L.) and non-netted honey dew fruit (*Cucumis melo* L. Inodorus group) combined, have surpassed bananas (*Musa* spp.) as the No. 1 most consumed fresh fruit in the U.S. (USDA, 2006). *C. melo*, in addition to their superior consumer preference, are an extremely healthful food choice as they are rich in ascorbic acid, β-carotene, folic acid, and potassium (Lester and Eischen, 1996; Lester and Crosby, 2002) as well as a number of other human health-bioactive compounds (Lester, 1997). Netted cantaloupes have a drawback: the netted rind is well-known to harbor human illness-related enteric bacteria such as *Salmonella* Lignieres (Castillo et al., 2004), *Shigella* Chatellani & Dawson (USFDA, 2003), and *Escherichia coli* O157:H7 (Del Rosario and Beuchat, 2004). Non-netted orange-fleshed honey dew (*C. melo* L. Inodorus group) is a suitable, commercially available, replacement for netted orange-fleshed cantaloupe. One cultivar of this fruit genotype, Orange Dew, is superior in β-carotene content, phenolic levels, and postharvest shelf life compared to netted cantaloupe (Hodges and Lester, 2006). Moreover, as a fresh-cut product 'Orange Dew' has lower ethylene and respiration rates and less microbial counts than orange-fleshed cantaloupe cubes, and consumers rated 'Orange Dew' fruit superior in flavor, texture, sweetness and overall eating quality (Saftner et al., 2006).

Orange-fleshed honey dew germplasm is generated by backcrossing green-fleshed honey dew with orange-fleshed cantaloupe or other β-carotene-rich melons. Breeding for

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orange-fleshed honey dew has been hampered by low consumer demand due to a lack of awareness of this fruit (K. Crosby, TX A&M, melon breeder, personal communication). Subsequently, only a few orange-fleshed honey dew cultivars and breeding lines are being tested in state extension variety trials for phytonutrient level and/or storage quality potential.

Antioxidants are important in relation to human health and play a crucial role in inhibiting the oxidative stress associated with senescence of fruits and vegetables (Hodges et al., 2004). In a recent study comparing malondialdehyde (MDA; an estimator of lipid peroxidation and hence oxidative stress) in netted cantaloupe 'Cruiser' with orange-fleshed honey dew melon 'Orange Dew', 'Cruiser' accumulated more MDA after 17-d storage than 'Orange Dew' (Hodges and Lester, 2006). Higher concentrations of β-carotene and activities of the antioxidant enzymes ascorbate peroxidase (AsPX), monodehydroascorbate reductase, dehydroascorbate reductase, catalase (CAT), guaiacol peroxidase, and superoxide dismutase (SOD) were observed in 'Orange Dew'.

In this study we compared antioxidants, minerals, and senescence quality indicators in orange-fleshed honey dew germplasm produced in different seasons and after 3–24 d storage at 5 °C or 10 °C. The overall objective of this study was to determine variation in enzymes and metabolites associated with both human health and fruit quality by assessing concentrations of compounds (ascorbic acid, β-carotene, folic acid, and total hydro- and lipophilic antioxidants), activities of enzymes (AsPX (EC 1.11.1.11), CAT, (EC 1.11.1.6), SOD (EC 1.15.1.1)), concentrations of minerals (calcium, iron, magnesium and potassium), and senescence quality indices (MDA and electrolyte leakage) in commercially available orange-fleshed honey dew cultivars: Honey Gold, Orange Delight, Orange Dew, and Temptation and one breeding.

2. Materials and methods

2.1. Plant material

Fruit of non-netted orange-fleshed honey dew (*C. melo* L. Inodorus group) cultivars Honey Gold (Harris Moran, Seed, Seed Co., Modesto, CA, USA), Orange Delight (Seminis Seed Co., Oxnard, CA, USA), Orange Dew (Shamrock Seed Co., Salinas, CA, USA), Temptation (Sakata Seed America Inc., Lehigh, FL, USA), and a breeding line (SVR-03935152, Seminis Seed Co.) were grown in a glasshouse following the procedures previously described by Lester et al. (2005). Briefly, plants were grown in 15 L black plastic pots containing a commercial potting medium [Sunshine mix #2 (Sun Gro Horticulture, Bellevue, WA, USA)] in a glasshouse at the U.S. Department of Agriculture, Agricultural Research Service, Kika de la Garza Subtropical Agricultural Research Center, Weslaco, TX (lat. 26° N, long. 97° W, elevation 21 m). Following germination (7 d after planting), seedlings were thinned to one per pot. Mutual shading among plants was minimized by placing pots at least 45 cm apart. Plants were watered at least once per day using an automatic drip irrigation system, and fertigated twice per week with a complete water-soluble fertilizer (10N–4.4P–8.3K, Peter's Corp., St. Louis, MO, USA) during vegetative and fruit developmental stages. During flowering/pollination, plants were fertigated twice per week with a 4.5N–9.9P–6.3K nutrient solution. Natural sunlight was supplemented with 400 W high-pressure sodium-vapor lamps. The average daily photosynthetic photon flux (PPF) at the canopy level was 20.7 ± 0.66 mol m^{-2} in spring and 12.3 ± 0.50 mol m^{-2} in autumn. Cumulative PPF for the entire growth periods were 1674 and 1238 mol m^{-2} for spring and autumn, respectively. Average day/night temperatures were 35.9 ± 0.7 °C/24.8 ± 0.3 °C and 29.9 ± 0.5 °C/22.8 ± 0.5 °C in spring and autumn, respectively, while average day/night RH values were 42 ± 1.4%/74.3 ± 1.1% and 51.2 ± 1.3%/74.0 ± 1.1% in spring and autumn, respectively. Flowers were hand-pollinated and only one fruit per plant was allowed to develop. Matured (abscised) fruit were harvested at 0800 each day.

2.2. Postharvest treatments

Following harvest, a group of fruit (10 replicates per treatment) was washed in distilled water and stored for 3 d at 21 °C to simulate retail display conditions prior to quality analysis. A second group of fruit was stored at 5 °C (autumn-grown) or at 10 °C (spring-grown) plus 95 ± 2% RH for 14 or 21 d to simulate commercial transport and storage conditions, followed by an additional 3 d at 21 °C prior to quality analysis. As a result, this second group of fruit was stored for a total of 17 or 24 d.

Following storage all fruit were washed in distilled water, the epidermis (peel) removed with a vegetable peeler, and the polar-ends (totaling two-thirds of the fruit) removed and discarded. Wedges of remaining equatorial-region mesocarp (pulp) tissue, devoid of seeds and integument tissue, were pureed in a food processor (Quick 'N Easy; Black and Decker, Towson, MD, USA) using 3- to 5-s pulses. Tissue samples were either (i) assayed fresh for malondialdehyde levels, electrolyte leakage, and antioxidant enzyme activities, (ii) frozen (liquid nitrogen, then stored at −80 °C) for ascorbic acid and folic acid content, or (iii) lyophilized (following freezing in liquid nitrogen) for β-carotene, minerals and sugar concentrations, and total antioxidant capacities.

2.3. Vitamin assays

Ascorbic acid and dehydroascorbate were extracted from 7.5 g frozen (−80 °C) tissue and determined according to the procedure of Hodges et al. (2001) and reported as total ascorbic acid. β-carotene was extracted under low light conditions from lyophilized tissue (0.020 g) using the procedure of Lester et al. (2005). Folic acid as 5-methyltetrahydrofolate was extracted from 7.5 g frozen tissue according to the procedure of Lester and Crosby (2002).

2.4. Metabolite assays

Malondialdehyde content was determined on 2.0 g of fresh pulp using the TBARS procedure of Hodges et al. (1999). Electrolyte leakage was determined on fresh pulp using three tissue

disks (10 mm × 1 mm thick) from three different regions along the fruit equatorial circumference incubated in 0.35 M mannitol solution and assayed as previously described (Lester and Stein, 1993).

2.5. Carbohydrates

Fruit sugars were extracted from 0.3 g lyophilized tissue by homogenizing in 5 mL 80% ethanol at 90 °C using a Polytron (Polytron, Kinematica, GmbH, Luzen, Switzerland) at speed #6 for 5 s, filtered (Whatman No.1; Maidestone, Great Britton) and the residue washed with 5 mL 80% ethanol at 90 °C. One mL of the combined filtrate was reduced to 0.2 mL at 50 °C under N_2, brought back to 1 mL with high-performance liquid chromatography (HPLC) grade water then passed through a pre-wetted (HPLC water) C18 Sep-Pak (Waters Corp., Milford, MA, USA) filter. Fructose, glucose and sucrose were quantified by co-chromatography with known standards for fructose, glucose and sucrose with refractive-index detection by HPLC using a Supelcogel Ca (30 cm × 7.8 mm i.d.) column equipped with a Supelcogel Ca guard column (Supelco, Bellefonte, PA, USA) heated to 80 °C and eluted with HPLC grade water at 8.33 μL s^{-1}. Tissue dry weight was determined as a percentage of fresh tissue after lyophilization.

2.6. Minerals

Calcium, iron, magnesium and potassium were extracted from 2 g lyophilized tissue by ashing (3 h at 550 °C) in acid washed porcelain crucibles. The cooled ash was dissolved in 2 mL 1.0 mol L^{-1} HCL, filtered (Whatman No.1; Maidestone, United Kingdom) and brought up to 100 mL with HPLC water. Calcium, iron, magnesium and potassium were calibrated against known standards using atomic absorption.

2.7. Enzyme assays

Activities of AsPX, CAT and SOD were analyzed in 15 g of fresh tissue as described in Lester et al. (2004).

2.8. Total antioxidant assay

Lipophillic and hydrophyllic antioxidants were analyzed using randomly methylated β-cyclodextrin as a solubility enhancer, 2,2′-azobis (2-amidino-propane) dihydrochloride as a peroxyl generator and 6-hydroxy-2,5,7,-tertamethylcroman-2 carboxylic acid (Trolox) as a standard according to Prior et al. (2003).

2.9. Statistics

Ten single plants per cultivar per storage time (100 plants total), with one melon fruit per plant, were used in both autumn and spring productions of this study. Data were subjected to analysis of variance using the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA). Treatment means were compared using the least square means (LSMEANS) procedure of SAS.

Table 1
Comparison of orange-fleshed honey dew breeding line (SVR-03935152) and commercial cultivars for levels of ascorbate, β-carotene, 5-methyltetrahydrofolate (folic acid), and for total sugars. Fruit were glasshouse grown in autumn 2005 and spring 2006. Fruit were stored 0, 14, or 21 d in autumn at 5 °C or in spring at 10 °C plus 95 ± 2% RH simulating commercial transportation/storage followed by 3 d at 20 °C to simulate retail display. All data are on a fresh weight basis

Genotype	Total storage (d)	Ascorbate (mg/kg)		β-carotene (mg/kg)		Folic acid (μg/kg)		Total sugars (g/kg)	
		Harvest		Harvest		Harvest		Harvest	
		Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
Honey Gold	3	171 BCa	150 Ba	12.5 Ba	10.2 Ba	446 Aa	573 Aa	82 Aa	72 Aa
Orange Delight	3	192 ABa	180 Aa	18.9 Aa	12.6 Aa	398 Aa	674 Aa	91 Aa	76 Aa
Orange Dew	3	224 Aa	181 Aa	18.2 Aa	15.5 Aa	393 Aa	695 Aa	96 Aa	76 Aa
SVR-03935152	3	139 Ca	161 Ba	8.1 Ca	10.6 Ba	291 Ba	573 Aa	85 Aa	65 Aa
Temptation	3	163 BCa	182 Aa	12.3 Ba	9.9 Ba	356 ABa	633 Aa	93 Aa	61 Aa
		Storage							
		5 °C	10 °C	5 °C	10 °C	5 °C	10 °C	5 °C	10 °C
Honey Gold	17	164 ABa	122 Ba	12.0 Ba	7.0 Bb	354 Aab	483 Aa	91 ABa	55 Bb
Orange Delight	17	181 Aa	172 Aa	13.2 ABb	12.0 Aa	353 Aab	553 Aa	97 Aa	71 Aa
Orange Dew	17	166 ABb	173 Aa	15.9 Aa	14.9 Aa	279 Aab	594 Aa	93 Aa	73 Aa
SVR-03935152	17	124 Ca	136 Bb	8.5 Ca	9.3 Ba	287 Aa	505 Aa	80 Ba	64 Ba
Temptation	17	146 BCa	135 Bb	10.3 BCa	8.6 Ba	320 Aab	497 Ab	80 Ba	61 Ba
Honey Gold	24	160 ABa	90 Bb	11.3 Ba	7.2 Bb	260 Ab	347 Bb	84 ABa	54 Ab
Orange Delight	24	172 ABa	131 Ab	13.4 Ab	10.9 Ab	273 Ab	489 Ab	96 Aa	62 Aa
Orange Dew	24	179 Ab	142 Ab	16.1 Aa	11.9 Ab	259 Ab	526 Ab	97 Aa	61 Aa
SVR-03935152	24	123 Ca	121 Ab	8.9 Ca	9.0 Ba	294 Aa	432 Ab	77 Ba	57 Ab
Temptation	24	142 BCa	99 Bb	10.9 BCa	6.5 Bb	266 Ab	300 Bb	75 Ba	51 Ab

Upper-case letters within a column indicate significant differences (LSMEANS 5% level) among cultivars within a storage period. Lower-case letters within a column indicate significant differences (LSMEANS 5% level) within a cultivar over storage time.

3. Results

3.1. Carbohydrates and vitamins

Production season affected orange-fleshed honey dew cultivars and breeding line fruit total vitamin C (ascorbate), pro-vitamin A (β-carotene), vitamin B9 (folic acid) and total fruit sugar contents (Table 1). Following a 3-d simulated retail display at 21 °C, the cultivars Honey Gold, Orange Delight and Orange Dew had higher levels of ascorbate in autumn compared to spring grown fruit while the breeding line and 'Temptation' demonstrated the opposite with lower ascorbate levels in the autumn. However, regardless of the production season, 'Orange Delight' and 'Orange Dew' were always among the cultivars with the highest ascorbate concentrations while the breeding line was amongst the lowest. There was a slight decline in ascorbate concentration during storage at 5 °C, with only 'Orange Dew' experiencing a significant decline. At 10 °C all germplasm fruit experienced a significant decline (Table 1).

β-carotene concentration in orange-fleshed honey dew fruit followed the same genotype, seasonal, and storage time by temperature effect as with ascorbate. 'Orange Delight' and 'Orange Dew' always had among the highest β-carotene concentration, with the breeding line among the lowest at harvest and throughout storage.

Folic acid concentrations in orange-fleshed honey dew fruit were higher (often nearly twofold) in the spring compared to autumn. All fruit from the spring harvest had similar concentrations of folic acid whereas in the autumn the breeding line had among the lowest folic acid content. Following 24-d storage at 10 °C folic acid in all fruit declined. Following 24-d storage at 5 °C, only the breeding line maintained a constant concentration of folic acid.

Total fruit sugar content following 3 d at 21 °C was higher in autumn compared to spring grown fruit. At harvest there was no significant difference in total fruit sugar content among the genotypes. Storage for up to 24 d at 5 °C resulted in no significant decline in sugar content within a genotype. Among genotypes, the breeding line and 'Temptation' fruit exhibited enough of a decline in sugar content at 5 °C to rank them significantly lower than 'Orange Delight' and 'Orange Dew'. Storage at 10 °C for 24 d demonstrated a significant decline in sugars in all fruit except 'Orange Delight' and 'Orange Dew'.

3.2. Minerals

Fruit calcium (Ca), iron (Fe), magnesium (Mg) and potassium (K) concentrations in orange-fleshed honey dew accumulated differently depending on genotype, growing season, and ion (Table 2). Calcium, unlike Fe, Mg, and K, accumulated in orange-fleshed honey dew fruit (nearly threefold) more in the spring than autumn. In contrast, Mg, K, and Fe levels were 5%, 12%, and 31%, respectively, higher in the autumn than in the spring. 'Orange Dew' in both seasonal production cycles had among the highest levels of Fe, Mg, and K compared to the other genotypes. Iron and K concentrations remained unchanged

Table 2
Comparison of orange-fleshed honey dew breeding line (SVR-03935152) and commercial cultivars for calcium (Ca), iron (Fe), magnesium (Mg) and potassium (K) concentrations

Genotype	Total storage (d)	Ca (mg/kg)		Fe (mg/kg)		Mg (mg/kg)		K (mg/kg)	
		Harvest		Harvest		Harvest		Harvest	
		Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
Honey Gold	3	46 Cb	212 Ab	3.7 Aa	2.7 Aa	113 Ba	127 Ba	2500 CDa	2490 ABa
Orange Delight	3	48 Ca	223 Ab	3.4 Aa	2.1 Aa	125 Bb	124 Ba	3030 BCa	2730 ABa
Orange Dew	3	85 Bb	188 Aa	4.9 Aa	3.7 Aa	151 Aa	141 Aa	3860 Aa	2870 Aa
SVR-03935152	3	42 Cb	165 Bb	3.8 Aa	2.5 Aa	131 ABa	126 Ba	2010 Da	2160 Ba
Temptation	3	98 Ac	133 Bc	4.1 Aa	2.8 Aa	134 ABa	105 Ca	2500 CDa	2050 Ba
		Storage							
		5 °C	10 °C	5 °C	10 °C	5 °C	10 °C	5 °C	10 °C
Honey Gold	17	62 Cb	270 Aa	3.0 Ba	2.3 Aa	99 Cab	103 Cb	2390 Ba	2190 Ba
Orange Delight	17	50 Ca	286 Aa	3.2 Ba	2.5 Aa	155 Aa	124 Ba	3380 Aa	2840 Aa
Orange Dew	17	86 BCb	193 Ba	5.8 Aa	3.5 Aa	161 Aa	141 Aa	3820 Aa	2960 Aa
SVR-03935152	17	103 Ba	257 Aa	3.1 Ba	3.4 Aa	110 Cb	106 Cb	2180 Ba	2060 Ba
Temptation	17	139 Ab	217 Bb	4.6 Aa	2.4 Aa	132 Ba	104 Ca	2700 Ba	2000 Ba
Honey Gold	24	131 Ba	273 Aa	2.9 Ba	2.4 Aa	123 CDa	106 Cb	2990 BCa	2260 BCa
Orange Delight	24	87 Ca	293 Aa	3.8 Ba	2.7 Aa	148 ABab	124 Ba	3280 ABa	2660 ABa
Orange Dew	24	136 Ba	199 Ba	5.0 Aa	3.6 Aa	161 Aa	148 Aa	3920 Aa	2970 Aa
SVR-03935152	24	136 Ba	279 Aa	2.6 Ba	2.1 Aa	107 Db	101 Cb	1950 Da	2080 Ca
Temptation	24	182 Aa	282 Aa	3.2 Ba	2.4 Aa	132 BCa	106 Ca	2650 CDa	2030 Ca

Fruit were glasshouse grown in autumn 2005 and spring 2006. Fruit were stored 0, 14, or 21 d in autumn at 5 °C or in spring at 10 °C plus 95 ± 2% RH simulating commercial transportation/storage followed by 3 d at 20 °C to simulate retail display. All data are on a fresh weight basis. Upper-case letters within a column indicate significant differences (LSMEANS 5% level) among cultivars within a storage period. Lower-case letters within a column indicate significant differences (LSMEANS 5% level) within a cultivar over storage time.

Table 3
Comparison of hydrophilic, lipophilic and total antioxidant capacities in orange-fleshed honey dew breeding line (SVR-03935152) and commercial cultivars

Genotype	Total storage (d)	Hydrophilic (Trolox μequiv./kg)		Lipophilic (Trolox μequiv./kg)		Total antioxidants (Trolox μequiv./kg)	
		Harvest		Harvest		Harvest	
		Autumn	Spring	Autumn	Spring	Autumn	Spring
Honey Gold	3	11.0 Ab	11.5 Ba	0.17 Ba	0.95 Aa	11.2 Ab	12.5 Ba
Orange Delight	3	11.7 Aa	11.5 Ba	0.53 Aa	0.81 ABa	12.1 Aa	12.3 Ba
Orange Dew	3	11.7 Ab	13.7 Aa	0.37 Ba	1.12 Aa	12.1 Ab	14.8 Aa
SVR-03935152	3	11.2 Aa	10.5 Ba	0.67 Aa	0.49 Ba	11.9 Aa	11.0 Ba
Temptation	3	10.5 Aa	9.9 Ba	0.41 ABa	0.53 Ba	10.9 Aa	10.4 Ba
		Storage					
		5 °C	10 °C	5 °C	10 °C	5 °C	10 °C
Honey Gold	17	13.3 Aab	12.5 Aa	0.31 Aa	0.66 Ba	13.6 Aab	13.2 Aa
Orange Delight	17	12.3 Aa	11.3 ABa	0.44 Aa	0.68 Ba	12.7 Aa	12.0 ABa
Orange Dew	17	10.8 Ab	13.7 Aa	0.36 Aa	1.21 Aa	11.2 Ab	14.9 Aa
SVR-03935152	17	12.1 Aa	9.2 Ba	0.55 Aa	0.44 Ba	12.7 Aa	9.6 Ba
Temptation	17	10.8 Aa	10.1 Ba	0.35 Aa	0.62 Ba	11.2 Aa	10.7 Ba
Honey Gold	24	15.7 Aa	13.2 Aa	0.16 Ba	0.61 Aa	15.9 Aa	13.8 ABa
Orange Delight	24	12.5 Ba	10.8 Ba	0.46 ABa	0.60 Aa	13.0 ABa	11.4 Ba
Orange Dew	24	14.8 Aa	13.8 Aa	0.72 Aa	1.00 Aa	15.5 Aa	14.8 Aa
SVR-03935152	24	12.7 Ba	10.8 Ba	0.38 Ba	0.81 Aa	13.1 ABa	11.6 Ba
Temptation	24	11.5 Ba	10.1 Ba	0.65 Aa	0.77 Aa	12.2 Ba	10.9 Ba

Fruit were glasshouse grown in autumn 2005 and spring 2006. Fruit were stored 0, 14 or 21 d at 5 or 10 °C plus 95 ± 2% RH simulating commercial transportation/storage followed by 3 d at 20 °C to simulate retail display. All data are on a dry weight basis. Upper-case letters within a column indicate significant differences (LSMEANS 5% level) among cultivars within a storage period. Lower-case letters within a column indicate significant differences (LSMEANS 5% level) within a cultivar over storage time.

during storage of the orange-fleshed honey dew fruit at 5 °C or 10 °C for up to 24 d. Magnesium generally remained unchanged by 24-d storage, but it declined in 'Honey Gold' at 10 °C and in the breeding line at 5 °C and 10 °C. Calcium was the only ion increasing in all genotype mesocarp following 24-d storage.

3.3. Antioxidants

Hydrophilic antioxidant capacity of orange-fleshed honey dew fruit was affected mainly by genotype; spring-grown 'Orange Dew' exhibited the highest capacity and 'Temptation' the lowest (Table 3). Lipophilic antioxidant capacity was mostly affected by growing season, with higher levels in the spring for all fruit except the breeding line. 'Orange Dew' fruit showed the greatest lipophilic antioxidant capacity difference (8.6-fold) between spring and autumn production. Total antioxidant capacities (a sum of hydrophilic and lipophilic capacities) were generally higher in spring-grown fruit. 'Orange Dew' had significantly higher total antioxidant capacities than 'Temptation' during both production seasons. Storage temperature and duration had virtually no effect on hydrophilic and lipophilic antioxidant capacities within or among the genotypes.

3.4. Enzymes

Following harvest of both spring- and autumn-grown fruits 'Honey Gold', 'Orange Delight' and 'Orange Dew' exhibited higher AsPX activities than 'Temptation' or the breeding line (Table 4). Although no changes occurred during storage at 5 °C, AsPX activity declined in both 'Honey Gold' and 'Temptation' following 24 d at 10 °C. AsPX activities were among the highest in 'Orange Delight' and 'Orange Dew' throughout storage at 5 °C and 10 °C.

Fruit CAT activities did not differ between the germplasm harvested in autumn, but spring-produced 'Orange Delight' had among the highest activities (Table 4). In general, CAT activities increased during storage at 5 °C in all fruit except 'Temptation' while no overall change occurred at 10 °C.

Fruit SOD activities were on average 2.3-fold higher in autumn than in spring (Table 4). Activities decreased in the breeding line during 5 °C storage, but increased in 'Orange Dew' when stored at 10 °C. Activities following 24 d at 10 °C remained unchanged for all fruit except 'Temptation' which increased, then decreased. Following 17 d and 24 d storage at 5 °C or 10 °C 'Orange Delight' and 'Orange Dew' consistently exhibited the highest SOD activities.

3.5. Metabolites

Malondialdehyde was higher in the spring-grown fruit compared with those produced in autumn (Table 4). Concentrations of MDA increased in all cultivars following 24 d at 5 °C except for 'Orange Delight' (5 °C) and 'Orange Dew' (5 °C and 10 °C). After 24-d storage MDA content of 'Orange Delight' and 'Orange Dew' fruit had increased 1.9 and 1.3, respectively, when stored at 5 °C, and 1.4 and 1.4, respectively, when stored at 10 °C. In contrast, the increase in MDA content during storage for the

Table 4
Comparison of ascorbate peroxidase (AsPX), catalase (CAT) and superoxide dismutase (SOD) activities, mesocarp malondialdehyde (MDA) content, and membrane senescence electrolyte leakage of orange-fleshed honey dew breeding line (SVR-03935152) and cultivars

Genotype	Total storage (d)	AsPX ascorbate oxidized (μmol/kg)		CAT H_2O_2 decomposed (mmol/(min kg))		SOD Cyt. c conserved (mmol/(min kg))		MDA (μmol/kg)		Electrolyte leakage (%)	
		Harvest		Harvest		Harvest		Harvest		Harvest	
		Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
Honey Gold	3	4.9 Aa	3.9 Aa	12 Ab	10 Ba	27 Ba	12 Aa	0.5 Bb	4.1 Ab	15 Aa	16 Aa
Orange Delight	3	5.0 Aa	4.3 Aa	17 Ab	24 Aa	28 ABa	14 Aa	0.8 Ab	5.7 Aa	17 Aa	20 Aa
Orange Dew	3	5.1 Aa	4.0 Aab	15 Ab	12 Ba	36 Aa	15 Ab	0.3 Ba	3.6 Aa	15 Aa	18 Aa
SVR-03935152	3	4.2 Ba	3.5 Ba	12 Ab	12 Ba	30 ABa	13 Aa	0.4 Bb	4.5 Ab	12 Ab	15 Ab
Temptation	3	4.3 Ba	3.8 Ba	15 Aa	16 Ba	24 Ba	9 Bb	0.4 Bb	3.4 Ab	13 Ab	19 Aa
		Storage									
		5 °C	10 °C	5 °C	10 °C	5 °C	10 °C	5 °C	10 °C	5 °C	10 °C
Honey Gold	17	5.1 Aa	3.5 Bb	27 Aa	7 Ba	28 Aa	9 Ca	0.6 Ab	6.8 Aa	16 Ca	22 ABa
Orange Delight	17	5.0 Aa	4.3 Aa	22 ABb	11 Ab	32 Aa	16 Ba	1.0 Aab	5.8 Aa	22 Ba	24 ABa
Orange Dew	17	4.6 ABa	4.1 Aa	21 ABa	7 Bb	33 Aa	20 Aa	0.3 Ba	3.7 Ba	20 BCa	19 Ba
SVR-03935152	17	4.4 Ba	3.1 Ba	20 ABab	10 Aa	27 Aab	12 BCa	1.0 Aab	6.9 Aa	26 ABa	26 Aa
Temptation	17	4.6 ABa	3.6 Bb	14 Ba	6 Bb	29 Aa	14 Ba	0.7 Aab	6.7 Aa	27 Ab	19 Ba
Honey Gold	24	4.7 Aa	3.5 Bb	24 Aa	8 Aa	22 Ba	10 Ca	1.5 Aa	8.5 Aa	19 Ba	23 ABa
Orange Delight	24	5.4 Aa	4.3 Aa	32 Aa	14 Ab	26 Ba	13 Ba	1.5 Aa	6.7 Aa	23 Ba	28 Aa
Orange Dew	24	5.0 Aa	4.4 Aa	22 ABa	13 Aa	38 Aa	20 Aa	0.4 Ba	4.7 Ba	21 Ba	25 ABa
SVR-03935152	24	4.1 Ba	3.2 Ba	20 ABa	9 Aa	18 Bb	9 Ca	1.8 Aa	10.9 Aa	29 Aa	28 Aa
Temptation	24	4.6 ABa	3.4 Bb	16 Ba	12 Aa	27 Ba	13 Bb	1.0 Aa	7.1 Aa	33 Aa	21 Ba

Fruit were glasshouse grown in autumn 2005 and spring 2006. Fruit were stored 0, 14 or 21 d in autumn at 5 °C or in spring at 10 °C plus 95 ± 2% RH simulating commercial transportation/storage followed by 3 d at 20 °C simulating retail display. All data are on a fresh weight basis. Upper-case letters within a column indicate significant differences (LSMEANS 5% level) among cultivars within a storage period. Lower-case letters within a column indicate significant differences (LSMEANS 5% level) within a cultivar over storage time.

other three genotypes ranged from 2.5 to 4.5 for fruit stored at 5 °C and 2.1–2.4 for fruit stored at 10 °C.

Electrolyte conductivity (electrolyte leakage) was similar between cultivars following 3 d at 21 °C. Leakage increased in the breeding line 59% and 46% at 5 °C and 10 °C, respectively, and increased 61% in 'Temptation' after 24 d storage at 5 °C. The other cultivars showed a non-significant increase in electrolyte leakage during storage (Table 4).

4. Discussion

Effects of cultivar, production soil type, fruit size, and year on ascorbate, β-carotene and sugar content in netted orange-fleshed cantaloupe and green-fleshed honey dew melon have been previously investigated (Lester and Eischen, 1996; Lester and Crosby, 2002). Our findings for sugars and vitamins corroborate those found in cantaloupe and green-fleshed honey dew, although concentrations trended higher in orange-fleshed honey dew fruit. An exact comparison cannot be made as these previous studies used fruits from melons commercially produced on sand or clay soil versus the glasshouse, soil-less potting mix, production used in the current study. Nevertheless, it is expected that orange-fleshed honey dew genotypes will accumulate these vitamins and sugars at levels substantially higher when grown on mineral soils (Saftner et al., 2006). As cantaloupes grown on clay soil were found to have higher sugars and vitamins than those grown on sandy soil (Lester and Eischen, 1996) a similar response is expected if orange-fleshed honey dew is grown on clay soil. The human bioavailability form of folic acid (5-methyltetrahydrofolate; Prior et al., 2003) is known to be affected by cultivar, fruit size, soil type and year (Lester and Crosby, 2002). In our study we found that season affected folic acid with spring-grown fruit having nearly twofold higher folic acid content than autumn-grown fruit (Table 1). As orange fleshed honey dew fruit is a cross between cantaloupe and green-fleshed honey dew, this seasonal effect on folic acid content may well occur in all *C. melo* fruit. Nevertheless, a complete understanding of the agronomic practices, climate, and seasonal regulation of folic acid in plants remains to be elucidated (Scott et al., 2000).

Calcium, Mg (Lester and Grusak, 1999) and K (Lester and Crosby, 2002; Lester et al., 2005, 2006) have been studied in melon, with K the only ion compared for influence of genotype (Lester and Crosby, 2002) and seasonal effect (Lester et al., 2006). Calcium is known to translocate within postharvest melon fruit from the peel to the seeds due to calcium's critical role in seed germination (Lester and Grusak, 1999). This loss of calcium from the peel through the pulp to the seeds is also linked to peel hypodermal-mesocarp plasma membrane leakage and to a hastened loss of fruit postharvest shelf life. Our data indicates that the translocation rate of Ca may be cultivar dependent. 'Orange Dew' exhibited a 1.1–1.6-fold increase in pulp Ca concentration by 24 d storage, whereas the breeding line exhibited a 1.7–3.2-fold increase.

Human dietary intakes of Ca, Fe, Mg and K ions in the U.S. is below the recommended daily values (Ca 1000 mg, Fe 18 mg, Mg 400 mg, and K 3500 mg) and consumers are encouraged to obtain these ions from a variety of fruits and vegetables rather than just from supplements (USDA, 2005). Cantaloupe and green-fleshed honey dew fruit are known to be relatively good to excellent sources of these dietary important ions when compared to the other top five fresh fruits [melons > bananas > citrus (*Citrus* spp.) > apples (*Malus x domestica*) and > grapes (*Vitis vinifera*)] most consumed in the U.S. (USDA, 2006). Fresh edible-tissue comparison of orange-fleshed honey dew genotypes for Ca, Fe, Mg, and K, averaged for all genotypes over spring and autumn production, versus the combined average for apples, bananas, citrus, and grapes gave orange-fleshed honey dew a concentration advantage (orange-fleshed honey dew combined mean vs. the other top five fruits combined mean, respectively: Ca 124 mg/kg vs. 110 mg/kg, Fe 3.4 mg/kg vs. 2.1 mg/kg, K 2620 mg/kg vs. 1970 mg/kg, and Mg 130 mg/kg vs. 120 mg/kg). Our data (Table 2) show that, on average, orange-fleshed honey dew fruit genotypes were equal (K) or exceeded (Ca, Fe and Mg) the Standard Reference values [and have from 1.5-fold to nearly 2.0-fold ion concentration variance making them suitable germplasm for further genetic improvement (K. Crosby, personal communication)]. Comparison of orange-fleshed honey dew genotype nutrient ion concentrations with USDA (2004) Standard Reference melon profiles may not be comparable due to highly dissimilar growing conditions.

A decline in postharvest quality is primarily associated with the development of senescence-related oxidative stress as well as decay (Hodges et al., 2004). Past work has implicated oxidative stress, defined as when the production of active oxygen species (AOS) such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$) exceeds the antioxidant capacity of the plant to scavenge them (Causin et al., 2006; Malacrida et al., 2006; Tewari et al., 2006). These AOS can target proteins, nucleic acids, and lipids; indeed, one of the major characteristics of oxidative stress is lipid peroxidation (Lacan and Baccou, 1998). Malondialdehyde (MDA), a secondary end-product of the oxidation of polyunsaturated fatty acids, provides an estimate of lipid peroxidation and hence oxidative stress (Hodges et al., 1999).

As antioxidants can scavenge/neutralize AOS, it has been hypothesized that tissue exhibiting high antioxidant capacities would better resist oxidative stress (and thereby delay senescence) than tissue with lower antioxidant potential. In comparing spinach cultivars with differing senescence rates, the cultivar with a lower senescence rate exhibited less rapidly declining activities of AsPX (which scavenges H_2O_2) and SOD (which scavenges $O_2^{\bullet -}$, leaving H_2O_2 as a by-product) and higher ascorbate content (Hodges et al., 2001; Hodges and Forney, 2003). In a previous comparison between an orange-fleshed honey dew melon and a netted cantaloupe melon, Hodges and Lester (2006), demonstrated that honey dew melon accumulated less lipid peroxidation and underwent less postharvest quality decline during storage than cantaloupe fruit. In our current study, orange-fleshed honey dew melon generally exhibited higher antioxidants such as β-carotene, and enzymes such as AsPX, CAT (which also scavenges H_2O_2) and SOD. These results suggest that the higher antioxidant capacity of orange-fleshed honey dew melon conferred a greater ability to defend against AOS than the netted cantaloupe fruit.

Malondialdehyde content increased in all melon cultivars during storage at both temperatures, but was higher in 'Orange Delight' fruit compared to fruit of the other germplasm. This cultivar along with 'Orange Dew' also consistently exhibited higher levels of total ascorbic acid and β-carotene and activities of AsPX, CAT, and SOD than 'Honey Gold', 'Temptation' and the breeding line. The higher activities of SOD would likely provide these two cultivars more capacity to dismutate $O_2^{\bullet -}$, while higher activities of AsPX and CAT would allow for more potential to remove the H_2O_2 resulting from higher SOD activity as well as from other metabolic sources. In comparing two non-netted muskmelon cultivars with differing storage life, Lacan and Baccou (1998) demonstrated that the cultivar exhibiting delayed senescence also had higher activities of CAT and SOD.

It is unclear why activities of AsPX, CAT, and SOD are higher at harvest and during storage in 'Orange Delight' and 'Orange Dew' compared to fruit of the other germplasm. As AOS can participate in cellular signaling which co-regulates antioxidant defenses (Fujita et al., 2006; Zhang et al., 2006), it is possible that 'Orange Delight' and 'Orange Dew' inherently produce higher amounts of AOS, are more sensitive to changes in AOS levels, and/or contain a more efficient cellular signal-gene expression system.

Although β-carotene levels were highest in 'Orange Delight' and 'Orange Dew', this was not reflected in the total lipophilic antioxidant capacity results. This is not surprising as in our previous study (Hodges and Lester, 2006) it was reported that equivalent concentrations of β-carotene standards provided approximately 5% of the total lipophilic antioxidant capacity values as did α-tocopherol using this AAPH and Trolox (water-soluble α-tocopherol analogue) system, thus underestimating carotenoid contribution to the total lipophilic capacity. In addition, although total ascorbate levels were highest in 'Orange Delight' and 'Orange Dew', this may have not been reflected in the total hydrophilic antioxidant capacity values. The ascorbate contribution to this value may have been diluted by that of other compounds such as phenolics, which consistently correlate with total antioxidant capacities as measured by this ORAC AAPH system (Kalt et al., 2001). Phenolic levels were not addressed in the present study.

Differences in photon flux densities between autumn and spring may explain some of the differences in fruit sugar, but ascorbic acid, and β-carotene accumulation between the two seasons. Photon flux levels (2070 and 1230 $\mu mol\ m^{-2}\ s^{-1}$ spring vs. autumn, respectively) were above the ~1200 $\mu mol\ m^{-2}\ s^{-1}$ but needed to saturate photosynthesis in most C_3 species such as melons. Excess light may result in photoinhibition of photosynthesis, but high temperatures, especially in spring-grown fruit would result in higher respiration rates in most of the spring grown germplasm fruit resulting in reduced sugar, ascorbic acid and carotenoid accumulation compared to autumn fruit (Lambers et al., 1998).

This study demonstrates there are germplasm (cultivar) differences in postharvest shelf life, vitamins, minerals, and antioxidants between non-netted orange-fleshed honey dew melon fruit. Our findings corroborate previous publications (Hodges and Lester, 2006; Saftner et al., 2006) that non-netted orange-fleshed honey dew melon is an excellent fruit option with both a long marketable shelf life and high nutrient content. Moreover, the lack of a netted rind and associated enteric fruit pathogen issues makes this a safer food choice relative to netted cantaloupe. As non-netted orange-fleshed honey dew melon germplasm is a relatively new fruit type to many melon producers, additional in-field evaluations are required to characterize/maximize consumer health and postharvest attributes.

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